

FIG. 1. Summary of meningococcal outer membrane vesicle vaccine efficacy trials *

Years of the Study	Vaccine Strain	Location (population vaccinated)	Age (years)	Estimated Efficacy (%)
1987-89	B:4:P1.15 C PS /alum	Cuba (100,000 school children)	11 to 16	83
1989-90	B:4:P1.15 C PSb/alum	Sao Paolo, Brazil (300,000 children)	2 to 4 4.1 to 7	47 74
1990-91	B:4:P1.15 C PS/alum	Rio de Janeiro, Brazil (2.4 million children)	3/12 to 7 4. to 7	58 71
1988-90	B:4:P1.3 C PS/alum	Iquique, Chile (40,000 children)	1 to 3.9 4 to 21	-23 70
1989-91	B:15:P1.7,16 alum	Norway (171,800 school children)	14. to 16	57

*Adapted from Frasch (1995, in MENINGOCOCCAL DISEASE, K. Cartwright (ed.), Wiley, New York, NY, p. 266). In Norway, two doses of vaccine were given separated by 6 weeks. Efficacy was 87% in the first year and then declined during the subsequent 18 months follow-up, so that overall efficacy was 57%. C PS=Serogroup C polysaccharide vaccine that is mixed with the vesicles. Alum preparations were Al(OH)₃

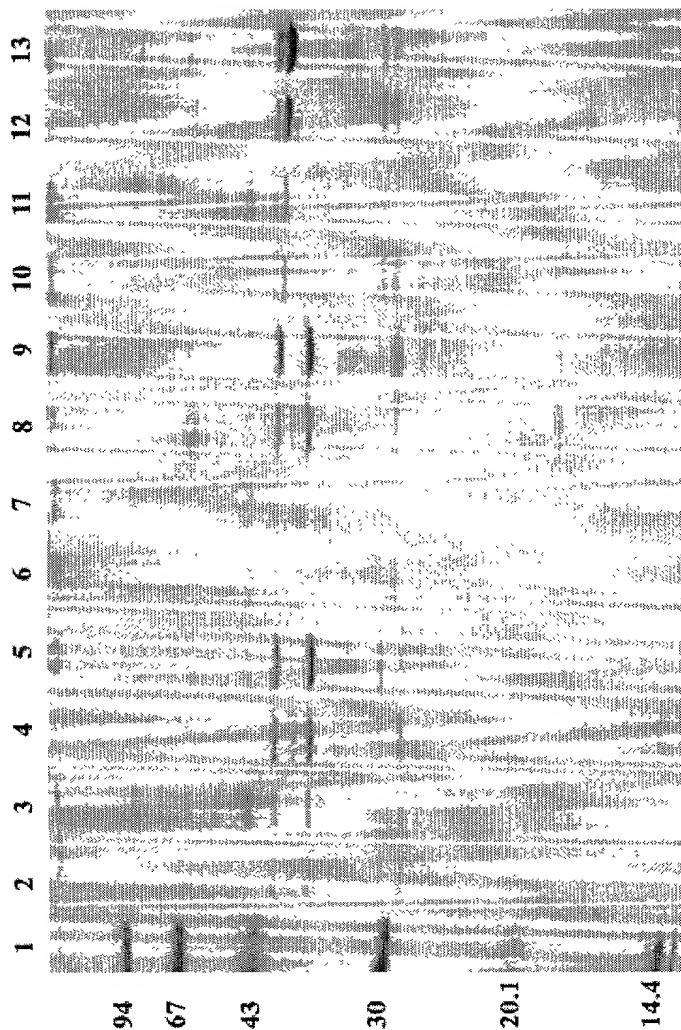


FIG. 2

FIG. 3.

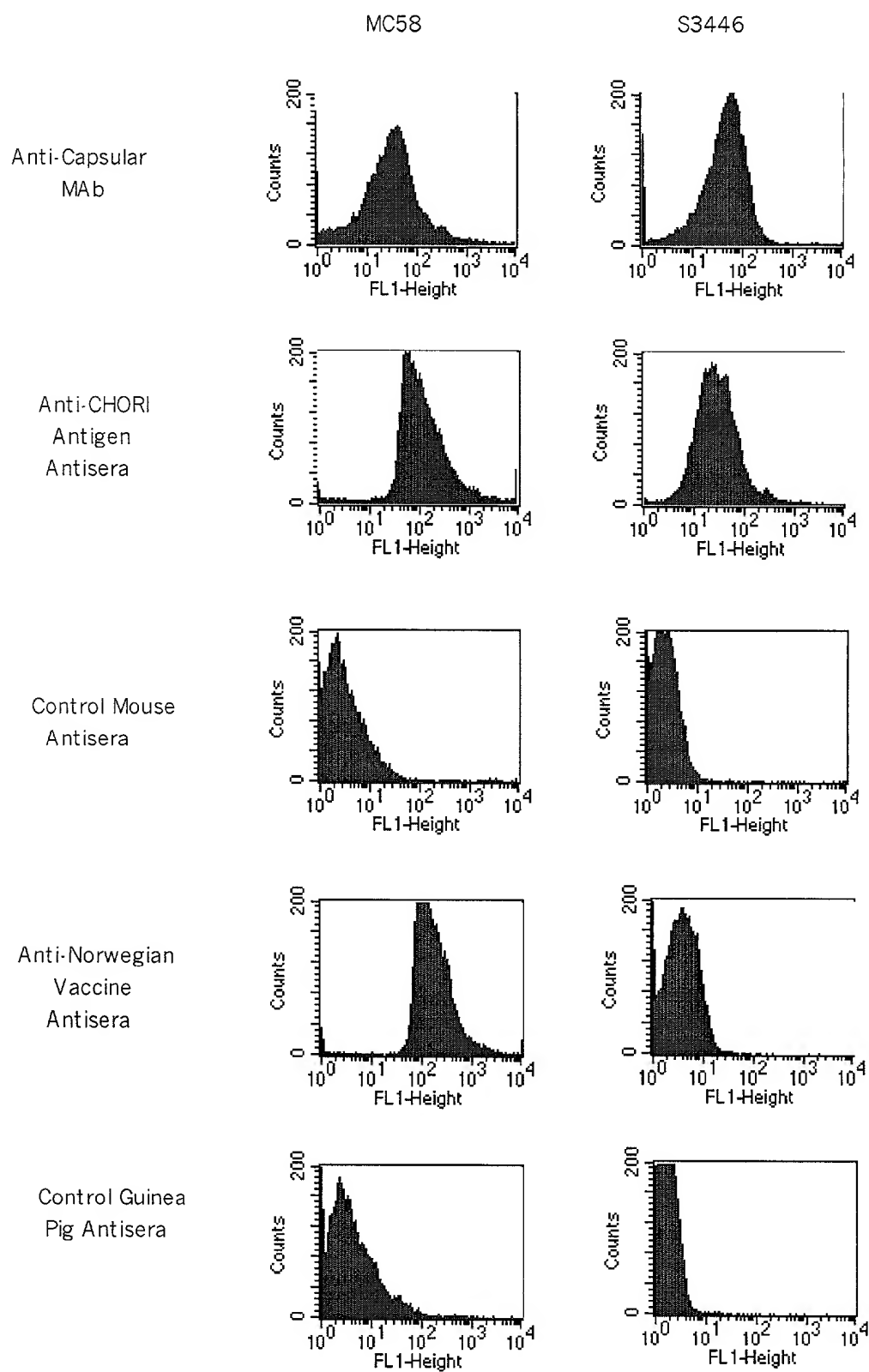


FIG. 4. Bacterial cell surface binding of antisera determined by indirect fluorescence flow cytometry

Strain (serosubtype)	PorA heterologous to CHORI vaccine strains ⁺⁺⁺	Surface reactivity by indirect fluorescence flow cytometry (1/titer) ⁺		
		Anti-CHORI ⁺⁺	Anti-Norway ⁺⁺	Anti-NspA ⁺⁺
1000 (5)	-	>200	>200	20
2996 (5,2)	-	2000	<20	20
8047 (5,2)	-	>200	<20	>20
BZ198 (4)	-	2000	200	>20
CU385 (19,15)	+	>200	<20	>20
IH5341 (7,16)	+	>200	>200	<20
M136 (P-)	+	<20	<20	<20
M986 (5,2)	-	2000	<20	<20
MC58 (7,16)	+	200	>200	<20
NG3/88 (1)	+	200	200	<20
NMB (.5,2)	-	2000	ND	>20
S3446 (23,14)	+	200	<20	<20

+Titer is defined as the dilution required to give 50% of fluorescence (FL1-Height) of 10 or greater over background fluorescence of cells in the presence of control sera.

++Anti-CHORI antisera prepared in mice by sequential immunization with MV from strain RM1090 (C:2a;P1.5,2), then with MV from strain BZ198 (B:NT:P1.4) followed by OMV from strain Z1092 (A:4,21;P1.10) (see text). Anti-Norway refers to antiserum from guinea pigs given two injections of OMV vaccine prepared from strain H44/76 (B:15:P1.7,16) by the National Institute of Public Health ("MenB-Folkhelsen"), Oslo, Norway. Anti-NspA refers to antisera prepared in CD1 mice given three injections of recombinant NspA as described by Moe et al. (1999 Infect. Immun. 67: 5664).

+++Serosubtype (PorA) differs from those of the three strains used to prepare the vaccine. (see FIG. 21 and 22).

FIG. 5. Reactivity of CHORI antisera against *N. meningitidis* serogroup A and C strains

Strain (serogroup:serosubtype)	Bactericidal Activity (1/titer)*		Surface binding by indirect fluorescence flow cytometry (1/titer) ⁺	
	Negative control sera	Anti- CHORI	Negative control sera	Anti- CHORI
60E (C:P1.7,1)	<10	>250	<20	~2000
Z1073 (A:P1.3,6)	<10	>250	ND	>>100

*Dilution of serum yielding 50% decrease in colony forming units (CFU) per ml after 60 minutes incubation of bacteria in the presence of human complement as compared to CFU/ml at time zero of controls (see FIG. 6 below).

⁺See footnotes to FIG. 4 and text

FIG. 6. Results of a bactericidal assay testing anti-CHORI antigen, anti-rNspA, and anti-Norwegian vaccine antisera against meningococcal B strain 2996

Complement	Animal source of sera or mAb	Antibody/Antisera ⁺	Final Concentration /Dilution	CFU/20 µl 0'	CFU/20 µl 60'	% Survival
None	-	None	0	189	250	132
None	-	None	0	171	250	146
Active	-	None	0	175	250	143
Inactive	-	None	0	180	250	139
Active	-	Complement	1:5	190	250	132
Active	Mouse	Anti-capsular mAb	200µg/ml		1	1
Active	Mouse	Anti-capsular mAb	100µg/ml		43	24
Active	Mouse	Anti-capsular mAb	50µg/ml		225	124
Inactive	Mouse	Anti-capsular mAb	200µg/ml		230	127
Active	Mouse	Anti-rNspA	1:10		250	138
Active	Mouse	Anti-rNspA	1:50		250	138
Active	Mouse	Anti-rNspA	1:250		250	138
Active	Mouse	Anti-CHORI antigen	1:10		0	0
Active	Mouse	Anti-CHORI antigen	1:50		2	1
Active	Mouse	Anti-CHORI antigen	1:250		55	30
Active	Mouse	Anti-E. coli control	1:10		>250	138
Active	Guinea pig	Anti-Norwegian vaccine	1:5		220	122
Active	Guinea pig	Anti-Norwegian vaccine	1:25		245	135
Active	Guinea pig	Anti-Norwegian vaccine	1:125		250	138
Active	Guinea pig	Anti-alum control	1:5		250	138

⁺See footnotes to FIG. 4 and text

FIG. 7. Complement-mediated bactericidal activity of antisera and antibodies.

Strain (serosubtype)	PorA heterologous to CHORI vaccine strains	Bactericidal activity (1/titer) ⁺			
		Anti-CHORI	Anti-Norway	Anti-NspA	
1000 (5)	-	130	>125	<10	
2996 (5,2)	-	>250	<5	<10	
8047 (5,2)	-	>250	<5	<10	
BZ198 (4)	-	>250	I ⁺⁺	110	
CU385 (19,15)	+	>250	<25	<10	
IH5341 (7,16)	+	>250	I ⁺⁺	<10	
M136 (P-)	+	<10	<5	<10	
M986 (5,2)	-	>250	<5	<10	
MC58 (7,16)	+	>250	>125	<10	
NG3/88 (1)	+	13	9	<10	
NMB (5,2)	-	>100	<5	16	
S3446 (23,14)	+	10	<5	<10	

+See footnotes to FIGS. 4 and 5 and text. Titer > refers to highest dilution tested; titer < refers to lowest dilution tested
 ++I, indeterminate due to the presence of bactericidal activity in the negative control antisera against this strain.

FIG. 8. Complement-mediated bactericidal activity of antisera from mice immunized with the indicated vaccines.

Strain (serosubtype)	PorA heterologous to CHORI vaccine strains ⁺⁺⁺	Bactericidal activity (1/titer) ⁺⁺⁺			
		CHORI CFA ⁺ (N=7)	CHORI/ Al ₂ (OPO ₄) ₃ ⁺ (N=7)	CHORI MIX/ Al ₂ (OPO ₄) ₃ ⁺ (N=10)	E. COLI MV/ Al ₂ (OPO ₄) ₃ (N=10)
1000 (5)	-	20	128	6	<4
8047 (5,2)	-	125	300	125	<25
BZ198 (4)	-	650	220	1000	<4
BZ198 NspA (4)	-	317	131	235	<4
BZ83 (10)	-	275	109	205	<25
CU385 (19,15)++	+	>128	128	<4	<5
H44/76 (7,16)	+	>128	>128	21	6
M136 (P-)	+	100	<4	5	<4
M986 (5,2)	-	193	101	133	<4
MC58 (7,16)++	+	47	8	7	<4
NG3/88 (7,1)	+	<4	4	<4	<4
NGP165 (5,2)	-	82	120	90	<4
NMB (5,2)	-	183	441	141	<4
S3032 (12,16)	+	125	400	230	<25
S3446 (22,14)	+	18	<4	<4	<4

+CHORI/CFA, sequential immunization with a 5 µg dose of strain RM1090 (C:2a;P1.5,2) MV with CFA, strain BZ198 (B:NT:P1.4) MV with IFA, and strain Z1092 OMV (A:4,21:P1.10) without adjuvant; CHORI/Al₂(OPO₄)₃ same as CHORI/CFA except using aluminum phosphate as an adjuvant; CHORI MIX/Al₂(OPO₄)₃, same as CHORI/ Al₂(OPO₄)₃ except each 5 µg dose contained an equal mixture of the three MV/OMV preparations; E. COLI MV/Al₂(OPO₄)₃ MV prepared from E. coli strain BL21(DE3).

++w/glu, cell culture grown in the presence of 0.3% glucose. ⁺⁺⁺ See footnotes and text of FIG. 4.

FIG. 9. Bactericidal activity of antisera from guinea pigs immunized with the indicated vaccines⁺.

Strain (serosubtype) ⁺⁺	PorA heterologous to CHORI vaccine strains ⁺⁺⁺	Bactericidal activity (1/titer) ⁺⁺⁺		
		CHORI/Al ₂ (OPO ₄) ₃ (N=8)	CHORI/Al(OH) ₃ (N=3)	E. COLI MV/Al ₂ (OPO ₄) ₃ (N=6)
M136 (P-)	+	<4	<4	<4
S3446 (22,14)	+	6	4	<4
CU385 (19,15)	+	12	5	<4
1000 (5)	-	64	16	<4
H44/76 (7,16)	+	64	16	<4
BZ83 (10)	-	24	12	<4
8047 (5,2)	-	>128	100	<4
BZ198 (4)	-	28	6	<4
BZ198ΔNspA (4)	-	19	5	<4
NG3/88 (7,1)++	+	9	5	<4

+CHORI/Al₂(OPO₄)₃, sequential immunization with a 5 microgram dose of strain RM1090 (C:2a:P1.5,2) MV, strain BZ198 (B:NT:P1.4) MV, and strain Z1092 OMV (A:4,21:P1.10) aluminum phosphate as an adjuvant; CHORI/Al(OH)₃, same as CHORI/Al₂(OPO₄)₃ except using aluminum hydroxide as an adjuvant; E. COLI MV/ Al₂(OPO₄)₃, MV prepared from E. coli strain BL21(DE3).

++All strains were grown in the presence of 0.3% glucose except for strains M136 and NG3/88.

+++See footnotes and text of FIG. 4.

FIG. 10. Passive protection in infant rats against meningococcal B strain 8047 bacteremia by antisera and antibodies.+

Treatment ⁺⁺	Dose/rat or Serum Dilution (100 microliters)	Blood Culture Obtained at 18 hrs	
		No. Positive/total no.	Geo. Mean, 10 ³ CFU/ml
Anti-Capsular mAb	10 micrograms	0/5	<1
PBS control	-	5/5	>200
Anti-E. coli control	1:20	5/5	>200
Anti-CHORI	1:20	0/5	<1
Anti-Norway	1:20	5/5	83
Alum control	1:20	5/5	178

+Animals were pretreated at time 0 with control or test antibodies and challenged 2 hours later with 5 x 10³ colony forming units of log phase N.meningitidis strain 8047 given IP.

++See footnotes to FIG. 4 and text.

FIG. 11. Passive protection in infant rats against meningococcal B strain 8047 bacteremia by guinea pig antisera.

Treatment ⁺	Dose/rat or Serum Dilution (100 microliters)	Blood Culture Obtained at 18 hrs	
		No. Positive/total no.	Geo. Mean, 10 ³ CFU/ml
Pre-immunization	1:10	6/6	21.9
Anti-CHORI/ Al ₂ (OPO ₄) ₃	1:10	0/6	<0.001
Anti-CHORI/ Al ₂ (OPO ₄) ₃	1:100	4/6	1.3
Anti-CHORI/ Al ₂ (OPO ₄) ₃	1:1000	6/6	193
Anti- CHORI/Al(OH) ₃	1:10	0/6	<0.001
Anti- CHORI/Al(OH) ₃	1:100	6/6	47.4
Anti- CHORI/Al(OH) ₃	1:1000	6/6	32.0
Anti-E. coli MV	1:10	6/6	110
Mouse anti-capsular mAb (SEAM 3)	20 µg	3/3	1.4

⁺See footnotes to FIGS. 9 and 10 and text.

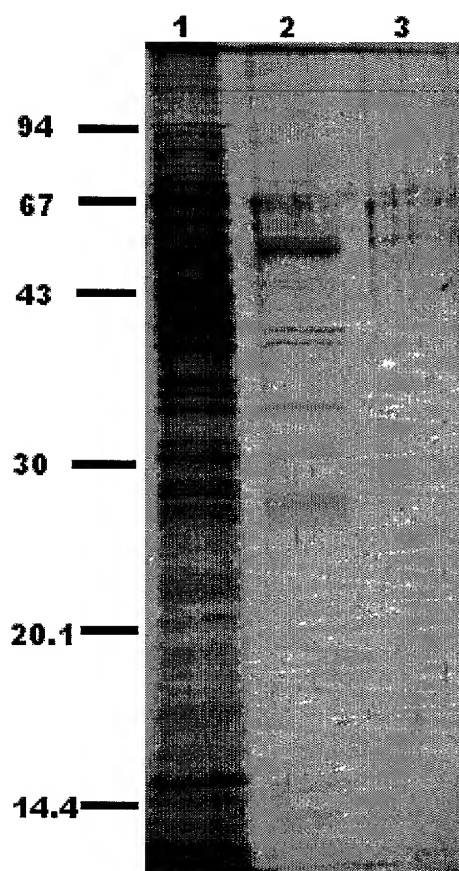


FIG. 12.

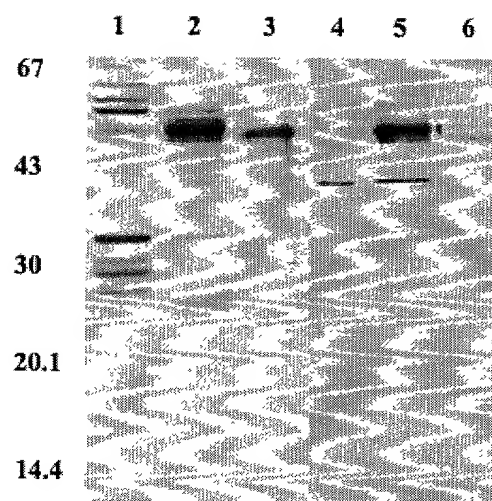


FIG. 13

FIG. 14. Bacterial surface accessible proteins precipitated by pooled antisera from mice sequentially immunized with MenC strain RM1090 MV, MenB strain BZ198 MV, and MenA strain Z1092 OMV.

Strain/Sample	Serotype:subtype [†]	Precipitated Proteins (by apparent mass in kDa)	
		37-41	31-33
RM1090MV	2a:P1.5,2:L3,7	40.7, 39.6	32
BZ198 MV	NT:NST	37.1, 35.1	32,30
Z1092 OMV	4,21:P1.10	40.7, 39.1, 38.6, 37.6	33.1, 32.5, 31.5
BZ198	NT:NST		32.5
CU385	4,7:P1.19,15		32.4
MC58	15:P1.7,16		32.9
NG3/88	8:P1.1		32.9
NMB	2b:P1.5,2		33
S3446	19,14:P1.23,14	40.7, 39.6	32.9
			14.5
			25.7
			14.5
			25.7

[†]See text and footnotes of Tables 21 and 22 (below)

FIG. 14A Bacterial surface accessible proteins precipitated by pooled antisera from mice sequentially immunized with MenC strain RM1090 MV, MenB strain BZ198 MV, and MenA strain Z1092 OMV.

Strain	Serotype:subtype ⁺	Precipitated Proteins (by apparent mass in kDa)							
		>45		36-45		25-35		<25	
		Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2
BZ198	NT:NST	80		36,39,43	37,40	28,30	28	14,5	12
CU385	4,7:P1.19,15	80		38,42	42	30,34		10	11
1000	NT:P1.5		ND	41, 45	ND	26	ND		ND

⁺See text and footnotes of FIGS. 21 and 22 (below). Expts 1 and 2 refer to experiments performed with immune serum pools from different groups of mice immunized with different CHORI vaccine preparations. ND= not done (strain 1000 tested with immune serum from one group of immunized mice).

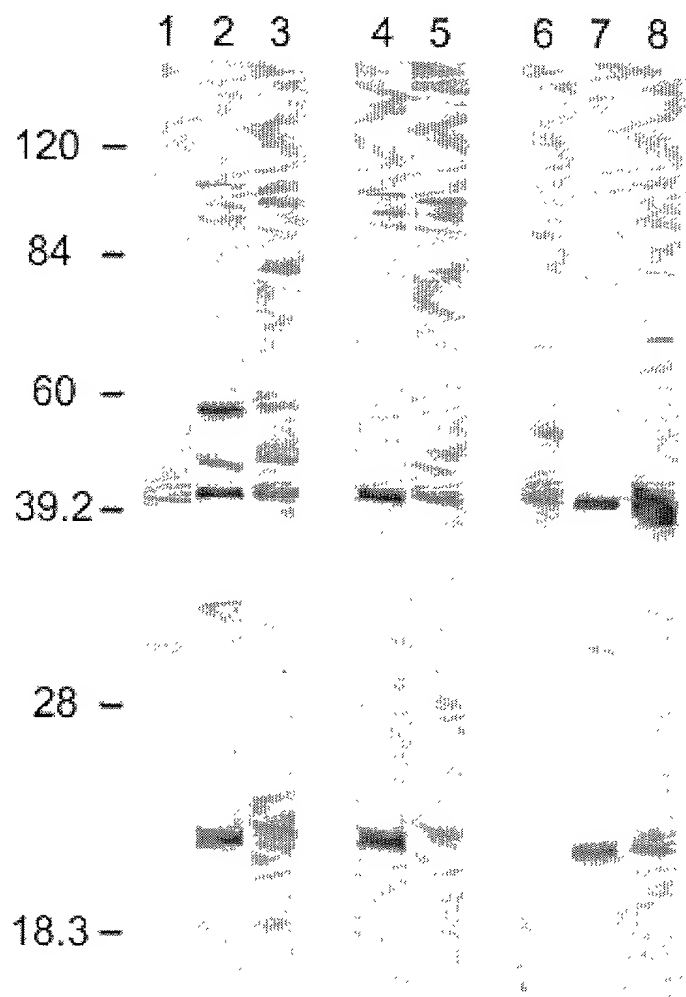


FIG. 15

FIG. 16. Proteins reactive with anti-CHORI antigen antisera by Western blot of MV and OMV preparations

Mouse anti-CHORI/CFA antisera ⁺		Mouse anti-CHORI Al ₂ (OPO ₃) ₂ ⁺		Guinea pig anti-CHORI/Al ₂ (OPO ₃) ₂ antisera ⁺⁺	
RM1090 MV	BZ198 MV	Z1092 OMV	BZ198 MV	RM1090 MV	Z1092 OMV
	119				
108					
101		101	101		
	96	97	95		
	93	93	91		92
		88			
		81			
		76			76
67	67	69		66	68
				62	64
53*	56*	57*	56	53	59
	50	50			
46*	47*	47*	47	47	46
	36	38			34
33*	33*	35*	33	32	33
	27			24	
	20*	21*	20		20
		19		19	19
	18*	18*	18		

⁺See footnote and text of FIG. 8.

⁺⁺See footnote and text of FIG. 9.

*Indicates proteins most reactive with CHORI/CFA antisera and common to at least two of the three vaccine preparations.

FIG. 17. Reactivity of anti-CHORI antisera with LOS by ELISA

Antisera ⁺	RM1090 LOS (1/titer) ⁺⁺		BZ198 LOS (1/titer) ⁺⁺		Z1092 LOS (1/titer) ⁺⁺	
	Unabsorbed	Absorbed ⁺⁺⁺⁺	Unabsorbed	Absorbed ⁺⁺⁺⁺	Unabsorbed	Absorbed ⁺⁺⁺⁺
Mouse anti-CHORI	<100	<100	900	<100	150	<100
Mouse anti-CHORI mix	900	100	200	<100	600	100
Guinea pig anti-CHORI	<100	<100	350	100	<100	<100
Guinea pig anti-CHORI mix ⁺⁺⁺	<100	<100	300	100	<100	<100

+ See footnotes and text of FIG. 8 and 9.

⁺⁺Titer is defined as the dilution of serum giving an OD 405 nm of 0.5 after 1 hr incubation with substrate.
⁺⁺⁺Same as used to prepare mouse anti-CHORI antisera mix (see FIG. 8) except that total dose of 25 micrograms protein was given rather than 5 micrograms.

⁺⁺⁺⁺After incubation with LOS-BSA coupled to Sepharose (see text).

FIG. 18. Bactericidal activity of anti-CHORI antisera before and after absorption of anti-LOS antibodies.

Serum ⁺⁺⁺	Strain BZ198 (1/titer) ⁺		Strain S3032 (1/titer) ⁺	
	Unabsorbed	Absorbed ⁺⁺	Unabsorbed	Absorbed ⁺⁺
Mouse anti-CHORI	49	28	259	247
Mouse anti-CHORI MIX	350	111	234	102
Guinea pig anti- CHORI	125	93	13	5
Guinea pig mix	77	31	<5	14

⁺See footnote and text to FIG. 5.
⁺⁺After incubation with LOS-BSA coupled to Sepharose (see text).
⁺⁺⁺See footnote and text to FIG. 17

FIG. 19. Reactivity of mAbs produced by immunization with CHORI vaccine with bacterial strains, LOS, and rNspA by ELISA.

Strain+	[mAb] (ng/ml) giving OD 405 nm = 0.5 after 1 hr incubation with substrate				Anti-NspA mAb AL4 ⁺⁺⁺ (IgG2a)
Nm 1000	1D9 (IgG2a)	4B11 (IgM)	9B8 (IgG3)	14C7 (IgG3)	1000
Nm 4335	500	>720 ⁺⁺	>3970	7380 ⁺	30
Nm 8047	500	>720	13	30	20
Nm 8047ΔNspA	200	2.4	>3970	10	>5400
Nm 8047ΔNspA	600	>720	>3970	>7380	20
Nm BZ198	600	>720	16	2	>5400
Nm BZ198 ΔNspA	600	>720	16	>7380	80
Nm BZ83	600	>720	>3970	273	180
Nm CU385	600	>720 ⁺⁺	>3970	>7380 ⁺⁺	400
Nm M136	600	>720	>3970	36	50
Nm M3966	1000	>720	>3970	0.5	400
Nm M986	800	>720	>3970	5	70
Nm NG3/88	600	0.8	>3970	1	100
Nm NGE31	500	>720	>3970	5	40
Nm NGF26	600	2.4	>3970	0.4	40
Nm S3446	500	>720	>3970	0.2	ND
Hi Minn A	>8300	>720	>3970	>7380	ND
Hi Eagan	>8300	>720	>3970	>7380	ND
Nm RM1090 LOS	>8300	>720	>3970	>7380	ND
Nm BZ198 LOS	>8300	>720	>3970	>7380	ND
Nm Z1092 LOS	>8300	>720	>3970	>7380	ND
rNspA MV	>8300	>720	>3970	28	6

+Nm, *Neisseria meningitidis*; Hi, *Haemophilus influenzae*
⁺⁺Although negative by ELISA, 14C7 is bactericidal for these strains (see FIG. 20).
⁺⁺⁺See Moe et al. Infect Immun. 2001 69:3762)

FIG. 20. Bactericidal activity of mAbs produced by immunization with CHORI vaccine.

Strain (serosubty pe)	PorA heterologo us to CHORI vaccine strains ⁺	mAb tested for bactericidal activity ⁺⁺				
		1D9	4B11	9B8	14C7	AL12 ⁺⁺ +
1000	-	-	+	-	+	+
BZ198	-	-	static	+	+	+
BZ198ΔN spA	-	-	-	+	-	-
CU385	+	-	+	-	+	-
M986	-	-	-	-	+	-
NG3/88	+	-	static	-	static	-

⁺See text and footnotes of FIG. 4.

⁺⁺ + refers to bactericidal when tested at less than or equal to 100 micrograms/ml; static refers to a percent survival of CFU/ml at 60 min is greater than 50% but less than 100% (see FIG. 6)

⁺⁺⁺ Moe et al. Infect Immun. 2001 69:3762

FIG. 21. Meningococcal serotype and serosubtype defining monoclonal antibodies available from RIVM*

Serotyping reagents			Serosubtyping reagents		
Monoclonal	Type	Ig	Monoclonal	Type	Ig
MN3C6B	1	G2b	MN14C2.3	P1.1	G2a
MN2D3F	2A	G2a	MN16C13F4	P1.2	G2a
MN2C3B	2B	G2a	MN20B9.34	P1.4	G2a
MN14G2I	4D	G2a	MN22A9.19	P1.1.5	G2a
MN5C8C	14	G2a	MN19D6.13	P1.6	G3
MN15A14H6	15	G2a	MN14C11.6	P1.7	G2a
			MN5A10F	P1.9	G2a
			MN5A10F	P1.9	G2a
			MN20F4.17	P1.10	G2b
			MN20A7.10	P1.12	G3
			MN24H10.75	P1.13	G2a
			MN21G3.17	P1.14	G3
			MN3C5C	P1.15	G3
			MN5C11G	P1.16	G2b

*Rijksinstituut Voor Volksgezondheid en Milieu (RIVM), P.O. Box 457, 3720 AL Bilthoven, The Netherlands (reference *FEMS Microbiology Letters* **48** (1987) 367-371).

FIG. 22. Serogroup, serotype, and serosubtype defining monoclonal antibodies available from NIBSC*

Serogroup	Cat. No.	Serotype	Cat. No.	Serosubtype	Cat. No.
A	95/674	P2.2a	95/682	P1.1	95/694
B	95/750	P2.2b	95/684	P1.10	95/710
C	95/678	P3.1	95/680	P1.12	95/712
		P3.14	95/688	P1.13	95/714
		P3.15	95/690	P1.14	95/716
		P3.21	95/692	P1.15	95/718
		P3.4	95/686	P1.16	95/720
				P1.2	95/696
				P1.3	95/698
				P1.4	95/700
				P1.5	95/702
				P1.6	95/704
				P1.7	95/706
				P1.9	95/708

*National Institute for Biological Standards and Control (NIBSC), Division of Bacteriology, Blanche Lane, South Mimms, Potters Bar, Herts., EN6 3QG, United Kingdom.